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THE DYNAMIC EFFECTS OF TREE SPECIES DIVERSITY ON NITROGEN IN SOIL

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An undergraduate thesis submitted in partial fulfillment of the requirements for the degree of Honours Bachelor of Science in Forestry

Faculty of Forestry and the Forest Environment,

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ABSTRACT

The availability of nitrogen in soil is one of the most important factors for tree growth. Although tree species mixtures are known to increase forest productivity, it remains unclear how they affect soil nitrogen. In this thesis, I focus on the effects of tree species diversity on soil resident nitrogen, particularly ammoniacal nitrogen (NH_4) and nitrate nitrogen (NO_3), through the review of 19 published papers. I also examined potential mechanisms associated with the influence of tree species diversity on soil nitrogen. I found a negative effect of tree species mixtures on the nitrogen concentration of soil. My goal was to evaluate the effects of tree species diversity on nitrogen in soil by comparing the soil traits of monocultures and species mixtures. I found that there were no significant differences in the effects on NO_3 and NH_4 in the soils of species mixtures compared to monocultures. My results suggested that despite increased nitrogen uptake by trees in species mixtures, the availability of nitrogen in soil did not decrease in species mixtures, which was likely due to increased nitrogen resorption, retention, and fixation.

KEYWORDS

Afforestation, nitrogen, soil, biodiversity, NO_3, NH_4, meta-analysis.

INTRODUCTION

Forests provide important resources for the production of timber, fuel, and pulpwood. Forest ecosystems also support many other functions, such as the prevention of soil erosion, carbon sequestration, preservation of biodiversity, and climate regulation. In Canada, forestry is a primary contributor to the economy, with the forest industry accounting for some 297,000 direct and indirect jobs. Except for functions related to ecology, forest ecosystems have myriad additional potential economic benefits in the provision of profitable lumber and plywood products, as well as a number of non-timber products including herbal medicines, fungus, livestock, etc.. The generation of these beneficial non-timber products have the capacity to effectively promote employment and improve the incomes of farmers, and are referred to as so-called "ecosystem services".

The development of forests has an intimate relationship with the macroelements and compounds of soils, of which nitrogen is a vital element that has specific effects on tree growth. Nitrogen is also an active element in the natural world, which is present in different forms that exist in the ambient atmosphere, soils, and organisms. These various forms of nitrogen are frequently able to transform into other forms. Fundamentally, nitrogen establishes a cycle in the soil, which includes nitrogen fixation, assimilation, ammonification, nitrification, denitrification, dissimilatory nitrate reduction to ammonium, anaerobic ammonia oxidation, and other processes. Nitrogen is absorbed into the soil, where it is taken up by plants, and then finally released back into the atmosphere as a gas. Nitrogen in the ambient atmosphere typically exists in its molecular state, where some nitrogenous compounds are also present. Free nitrogen in the atmosphere cannot be directly absorbed and utilized by higher plants; however, it can be fixed by nitrogen-fixing microorganisms to become combined nitrogen that is absorbed by plants and soil systems. Many tree species have the ability to fix nitrogen in the soil, while influencing changes in nitrogen levels therein. Instances of tree mixtures with nitrogen-fixing species that had the capacity to improve the growth of non-nitrogen-fixing species have been found in a number of peer-reviewed research reports (Luo, et al., 2016; Gunina, et al., 2017). For example, black locust (*Robinia pseudoacacia*), which is an N-fixing legume tree, has a strong capacity to increase soil N levels, thereby facilitating the growth of non-nitrogen-fixing species (Rice et al., 2004; Tateno et al., 2007).

In recent decades, the scope of afforestation has rapidly increased on a global scale (Berthrong et al., 2009). The effects of global environmental changes on soil nitrogen pools and fluxes have consequences for ecosystem functionality, such as plant productivity and N retention (Mueller et al., 2013). Nitrogen contributes significantly to plantations, where changes in soil N stocks are strongly correlated and have similar temporal patterns (Fan and Yang, 2016). Significant N stock increases were found 30 and 50 years following afforestation (Orwin and Wardle, 2005). Prior to these time points, N stocks were either depleted or unchanged, and soil N stocks were observed to increase in subtropical zones. They were also found to increase following afforestation with hardwoods (excluding Eucalyptus), and decreased after afforestation with pine (Li, et al., 2012).

One of the reasons that minerals in soils are reduced is due to the removal of native plants to meet the requirements of agricultural purposes worldwide. A large proportion of the world's soils are exposed to erosion to various degrees. Soil erosion related to vegetation may be divided into three aspects: (i) stems and leaf vegetation can reduce the kinetic energy of raindrops; (ii) stems, dead branches, and leaves can slow down runoff flow rates; and (iii) root systems can improve resistance to soil erosion (Leloup, 2018). Soil erosion is the primary reason for nitrogen loss in soils, where vegetation affects nitrogen loss by reducing erosion. At present, many scholars have focused on the study of sediment nutrient filtration in grassland belts; however, the relationships between vegetation and soil erosion, nitrogen enrichment, and loss has not been reported to date (Bi et al. 2007). Millions of tons of fertile surface soils are lost through erosion annually. Once soil erosion becomes severe, the minerals that are important for vegetation can be very rapidly lost. Depending on how sustainably harvested biomass is managed, its frequent removal can also deplete soil nutrients from these ecosystems, lower primary productivity of future rotations, and reduce their long-term potential as carbon sinks (Bi et al. 2007). One of the most common methods adopted worldwide for combating erosion is afforestation (Selma, 2014). If forests are properly managed, or reforestation efforts are conducted, numerous soil properties including nitrogen and SOC can potentially recover.

Soil properties, the development of AM fungi symbiosis, and plant nutrition play significant roles in tree productivity. Mixed plantations may derive from afforestation-type induced changes in soil properties and plant nutrition (Chen et al., 2017). The productive potential is increased in mixed stands, in contrast to pure stands, which is primarily due to the greater availability of nitrogen in the soil. This is provided by accessory species, as the availability of nitrogen is typically the most limiting factor for plant growth in temperate forests. Some research indicates that productivity and availability are associated with soil resident microbial biomass (Li et al., 2004). There is increasing evidence that microbial attributes might be employed as potential indicators of the impacts of forest management practices on soils (Mendham et al., 2002).

Soil is vital to plantations via the nitrogen cycle. If afforestation is improperly managed, rapidly growing plants and frequent harvesting could deplete nutrients and degrade soils. Nevertheless, the magnitude and direction of soil carbon accumulation following afforestation and its regulation by soil nitrogen dynamics are still not well understood (Cohen, 2013). My objective for this meta-analysis was to investigate the effects of tree species diversity on soil nitrogen. This study examined the effects of monocultures and species mixtures through a formal meta-analysis of nitrogen changes, which focused on inorganic N pools, including NH_4 and NO_3, with further data collected from globally distributed sites.

Material and Methods

Data collection

Data resources on the effects of biodiversity on nitrogen in soil were collected from scientific journals. Some of these journals were mainly focused on the insight of nutrient cycling between monocultures and mixtures, while others generally discussed the effects of afforestation on soil properties and diversity. The final data set contained papers that explored the relationships between vegetation and soils.

Various traits were collected and classified from these papers, which focused on specific nitrogen species and how they were altered with and without treatments. Through meta-analysis, we can identify the primary effects that afforestation has on the proportion of nitrogen in the soil. Different types of vegetation were involved in the various experiments. For instance, some experiments were conducted between conifers, broadleaves and their mixtures, while others were conducted between broadleaves, nitrogen-fixing species, and their mixtures.

Table 1. Studies included in this meta-analysis (complete citations shown in Literature Cited section)

Reference	Country	Eco type
Ali Bagherzadeh, et al. (2008)	Germany	Forest
Anna Gunina, et al. (2016)	The U.K.	Forest
Bin Hu, et al. (2016)	China	Forest
Da Luo, et al. (2016)	China	Forest
David A. Wardle, et al. (2000)	New Zealand	Grassland
David Rivest (2015)	Canada	Forest
E.L. Pereira, et al. (2011)	Portugal	Forest
Johanna Pausch, et al. (2012)	The U.S.	Greenhouse
Jinliang Liu, et al. (2018)	China	Forest
Julie Leloup, et al. (2018)	Europe	Greenhouse
Kevine Mueller, et al. (2013)	America	Grassland
K.H. Orwin and D. A. Wardle (2005)	New Zealand	Grassland
Pascal A. Niklaus, et al. (2005)	New Zealand	Grassland
Simone Cesarz, et al. (2013)	Germany	Forest
Shi Fan and Ning Yang (2016)	China	Forest
Thais Rodrigues Coser, et al.(2016)	Brasil	Cropland
Xuedong Chen, et al. (2017)	China	Forest
Y. Huang, et al. (2004)	China	Forest
Yumei M, et al. (2010)	China	Forest

For each site, we extracted the values, for instance, objective conditions including temperature, location, ecotype, the values of controlled and treated NH_4 and NO_3, total mineral nitrogen, the number of replications, etc. All data was collected directly from the tables provided in the article, or by using PlotDigitizer to digitally extract these data from figures.

Statistical Analysis

The InRR (natural log-transformed response) was employed to quantify the effects of plant mixtures or litter mixtures following Hedges, Gurevitch, and Curtis, (1999):

$$lnRR = \ln\left(\frac{\overline{X_t}}{\overline{X_c}}\right) = \ln\overline{X_t} - \ln\overline{X_c} \tag{1}$$

In this equation, $\overline{X_t}$ and $\overline{X_c}$ are the observed values of selected variables in the mixture, and the expected values of the mixture in each study, respectively. We computed the lnRR as the "effect size", which improved its statistical behavior in the meta-analyses. The $\overline{X_c}$ was also calculated based on weighted values of the component species in monocultures, following Loreau and Hector, (2001):

$$\overline{X_c} = \sum(p_i \times m_i) \tag{2}$$

In this equation, p_i is the observed value of the selected variable of species i in monocultures or single-species litter treatments, and m_i is the proportion of species i density in mixed forests, grasslands, croplands, and greenhouse pots. When there were multiple types of mixtures and experiment durations reported in a study, $\overline{X_C}$ and $\overline{X_t}$ were calculated separately for each mixture type and experimental year. This strategy accounted for the effects of species composition and plant quality differences between the mixtures and monocultures that corresponded to each mixture type and stand age within each original study.

Estimates of the effect sizes and subsequent inferences in the meta-analyses could be related to how individual observations were weighted. This is because weightings based on variances of samplings could significantly affect only a few individual observations. Therefore, the average *lnRR* was determined predominately by a small number of studies. Subsequently, we employed the number of replications for weighting:

$$W_r = \frac{(N_c \times N_t)}{(N_c + N_t)} \tag{3}$$

where W_r is the weight associated with each *lnRR* observation, and N_c and N_t are the numbers of control and treatment replications, respectively.

For my tests, I set the focus on the ln*RRs* of NO_3 and NH_4 associated with plant diversity. I employed the following model to determine the overall effects of SR, stand age, or experimental period (SA years) and their interactions:

$$\ln RR = \beta_0 + \beta_1 \cdot \ln(SR) + \beta_2 \cdot \ln(SA) + \beta_3 \cdot \ln(SR) \times \ln(SA) + \pi_{\text{study}} + \varepsilon \qquad (4)$$

where β, π_{study}, and ε are coefficients, the random effect factor of “Study”, and sampling error, respectively. The random effect explicitly accounted for autocorrelation between observations within each “Study”. I conducted the analysis using a restricted maximum possibility estimation with the *lme4 1.1-19* package (Bates et al., 2017). When continuous predictors, that is, ln(*SR*) and ln(*SA*) in Equation 4, are centered or scaled (minus mean and divided by one standard deviation), β_0 is the overall mean ln*RR* at the mean ln(*SR*) and ln(*SA*) (Cohen & Alken, 2013). To facilitate comparisons between NH_4 and NO_3 that had variable ln(*SR*) and ln(*SA*), I scaled these variables in my analysis. All statistical analyses were performed in R 3.5.3.

Results

In this meta-analysis study, the physical and chemical properties of soils collected from public resources demonstrated that these treatments were very similar, since there were significant differences ($p<0.05$) in NO_3 and NH_4 in the soils between monocultured plantations and mixtures (see Fig. 1).

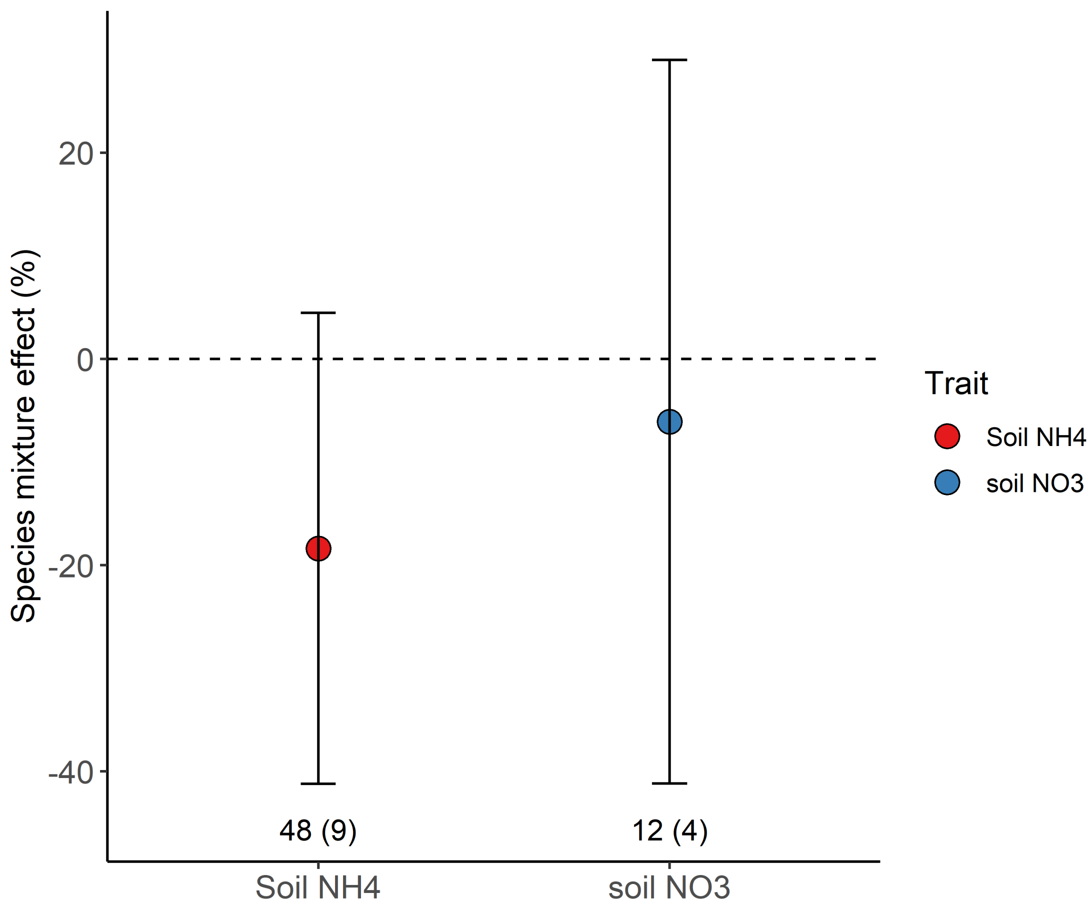

Figure 1. Species mixture effects on soil NH_4 and NO_3. This indicates a non-significant difference ($p<0.05$) of the species mixture effects on soil NH_4 and NO_3 between monocultures and species mixtures with 95% confidence intervals.

We can see from Fig. 1 that species mixtures can decrease the content of both NH_4 and NO_3. The soil NH_4 decreased by ~18%, whereas the NO_3 decreased by only ~5%. However, this difference caused by mixture species was not significant because these two points in Fig. 1 did not cover the line of "0", which means that compared to monocultures, the mixtures had no significant differences in their effects on NO_3 and NH_4. Both monocultured and species mixtures caused the reduction of nitrogen in the soil, nevertheless, according to this global meta-analysis the extent to which monocultured and species mixtures reduced nitrogen was similar. The treatment effects were significant at $\alpha = 0.05$ if the 95% CIs did not cover zero.

Discussion

As anticipated, my analysis revealed a non-significant difference in effects on NO_3 and NH_4 in the soils between monocultures and species mixtures. This meta-analysis revealed that species mixtures had similar effects on nitrogen in soil to monocultures from a global-scale perspective. Greater plant diversity led to higher plant and soil microbial activity (Chen and Chen, 2019). Further, some research indicated that the higher growth rates of these exotic plantations might lead to more drastic changes, such as negative impacts on water balance, soil fertility, and native biodiversity than did plantations that employed native tree species (Hofstede et al., 2002).

On the surface, the quantity of nitrogen in the soil dropped, as its uptake outpaced the supply rate, which increased leaching to groundwater, or led to decreases in mineral weathering (Binkley, 1995). The continuous reduction of nitrogen in the soil is detrimental for aboveground afforestation, which is also related to exchangeable cations and sodium. In many countries, logging residues are typically removed or burned following harvesting, or before subsequent rotations are planted, which leads to significant carbon and nitrogen losses. Therefore, we suggest that on some sites, if necessary, the logging residues can remain to improve the nitrogen and carbon levels of the soils, in case they are too depleted during the forest operations. One study indicated that microbes immobilize more nitrogen in their biomass as the C:N ratio increases, and as a consequence mineralization rates are lower, which leads to lower available nitrogen for plants, with subsequent lower productivity (Cong, et al., 2014). It was also found that some mixed stands with nitrogen-fixing secondary species may improve the vigor and stand environment of targeted species.

Conclusion

For this global meta-analysis study, the effects of species mixtures on soil NO_3 and NH_4 were very similar to monocultures. There were no significant differences ($p<0.05$) in the effects on NO_3 and NH_4 in soils between monocultures and species mixtures according to our results. The proper management of soil is required as rapidly-growing plants and frequent harvesting can deplete nutrients and degrade soils. This global meta-analysis also indicated that afforestation often leads to more acidic and nutrient deficient mineral soils; however, best management practices can assist to some extent with overcoming some of these changes and challenges Although these soil changes might impair the productivity of successive rotations, it remains unclear how long it will take to observe noticeable declines in productivity (Hofstede et al., 2002).

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